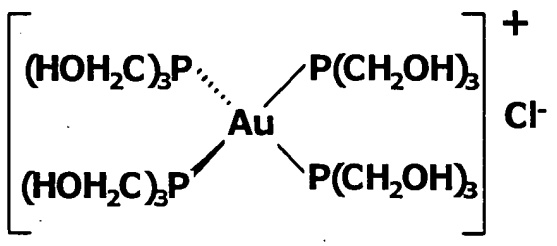
In vitro/In vivo Antitumor Properties of MU-Gold

An important determinant for biological evaluation of metal-based compounds is whether they are stable in solution and especially under the testing conditions. Gold compounds, in general, often react with thiol groups present in serum and cell culture media and this will result in reduction of cytotoxicity.



MU-Gold

MU-Gold is undoubtedly unique because our detailed *in vitro* and *in vivo* studies,including *in vivo* pharmacokinetic studies using the radiolabeled Gold-198 analogue of MU-Gold in rats, have demonstrated that it maintains its structural integrity and is not decomposed *in vivo*. In fact, MU-Gold is one of the rare examples of a water soluble tetrahedral coordinated Au(I) compound with excellent kinetic stability under *in vitro* and *in vivo* conditions. MU-Gold can be produced in 10-50 g quantities as a >99.99% pure compound and its aqueous (and NaCl) solutions can be stored without fear of decomposition over 12 month periods.

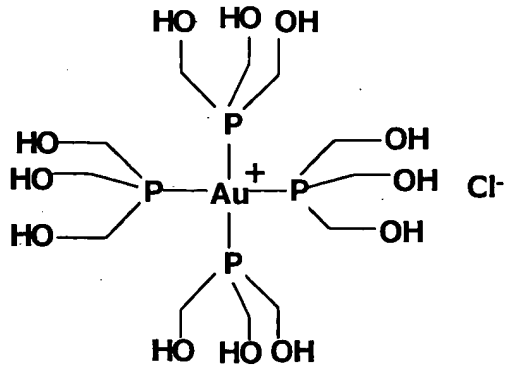
At the current time, there are no chemotherapeutic agents available that can effectively control the growth and metastasis of human androgen independent prostate cancer cells. In this context, new agents that decrease the rate of proliferation either directly or by increasing the rate of programmed cell death, allowing for improved treatment of androgen-independent disease are clearly needed. Our recent investigations on MU-Gold have demonstrated its excellent efficacy in suppressing the growth of HCT-15 cells (derived from human colon carcinoma), AGS cells (derived from human gastric carcinoma), androgen dependent LNCaP and androgen independent PC-3 cells (derived from human prostate carcinoma) (see Preliminary Studies Section). The remarkable activity of MU-Gold in slowing the growth of androgen dependent (LNCaP) and androgen independent (PC3) cells is of particular significance for its potential use in the treatment of hormone refractory prostate cancer. It is also of paramount importance that MU-Gold has demonstrated unique selectivity in elongating (or arresting) G1 phase of the cell cycle. With this cell cycle specificity, MU-Gold can be potentially used in combination with other chemotherapeutic agents that are cytotoxic to this specific phase (G1) for a greater combined efficacy.

Therefore, the potential clinical applications for MU-Gold in the treatment of prostate and various other cancers are great. As part of our research effort on the design and development of metal and radiometal based cancer diagnostic and therapeutic agents, we have recently investigated the *in vitro* and *in vivo* antitumor properties of a novel tetrahedrally coordinated [tetrakis(tris-(hydroxymethylphosphine))gold (I)] cation $[Au(P(CH_2OH)_3)_4]^+$ (MU-Gold) (Figure 1). MU-Gold was discovered in our laboratory *via* ligand displacement pathway using aqueous-organic biphasic solvent combination in 85% yields (Scheme 1).

An important objective of our preliminary investigation was to assess the efficacy of MU-Gold toward suppressing growth of cells derived from androgen dependent and androgen independent prostate carcinoma. Our preliminary studies of *in vitro* cell suppression by MU-Gold demonstrated marked activity against HCT-15 cells (derived from human colon carcinoma), AGS cells (derived from human gastrointestinal carcinoma), LNCaP cells (derived from androgen dependent human prostate carcinoma), and PC-3 cells (derived from androgen independent human prostate carcinoma cell lines) (Table 1). The remarkable efficacy of MU-Gold in suppressing growth of a wide variety of tumor cells at extremely low doses is unprecedented for metal-based chemotherapeutic agents.

The cell suppression efficacy (Table 1 and Figure 2) against androgen dependent (LNCaP) and androgen independent (PC3) cells is of particular significance in the quest towards the development of new treatment modalities for prostate cancer. Our work in the development of this novel chemotherapeutic agent with demonstrated efficacy against prostate cell lines *in vitro,* has potential to improve quality of life and longevity in prostate cancer patients.

Figure 1



Tetrakis(tris-(hydroxymethyl))gold chloride (MU-Gold)

Scheme 1: Biphasic Reaction for the Synthesis of MU-G ld

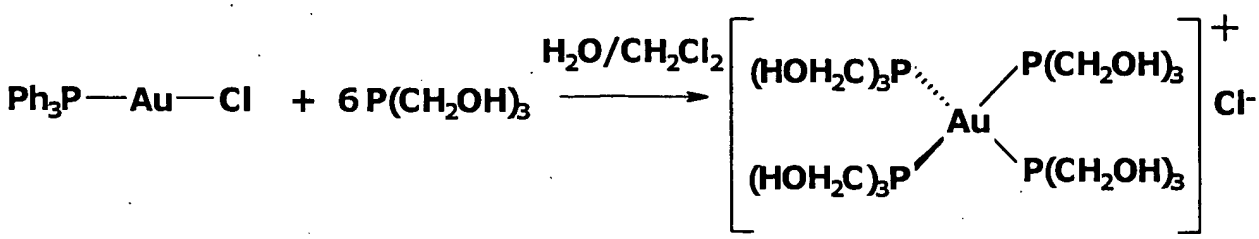
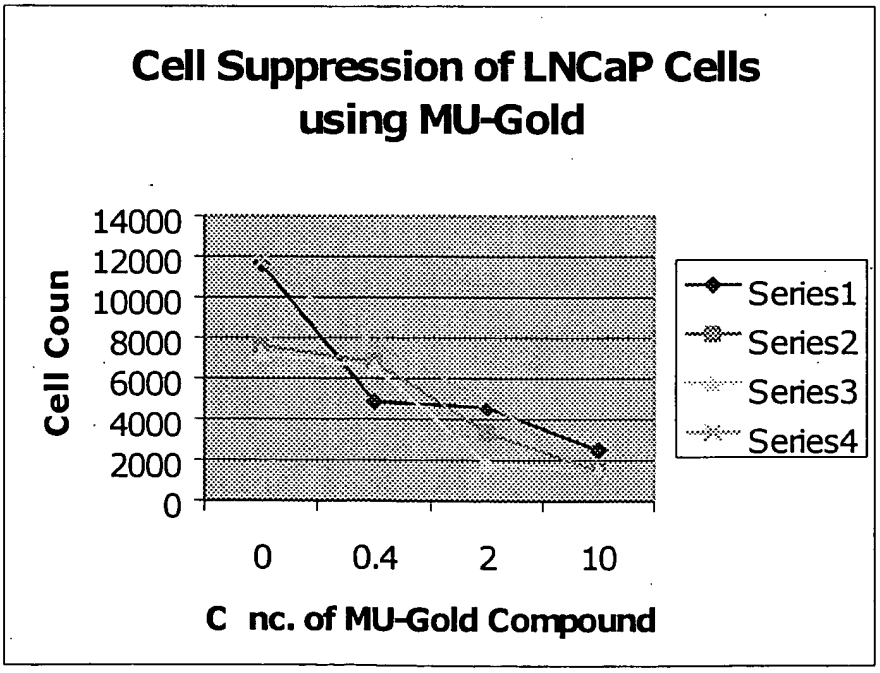


Table 1: Suppressive Effects of MU-Gold Cultured Cell Growth

Cell Type	Cell suppression data with different concentrations of MU-Gold		
	10 μg/ml	2 μg /ml	0.4 μg/ml
LNCaP	**98.32±1.2%**	**95.10±3%**	**93.24±4.7%**
HCT-15	82.82±7.8%	40.74±10%	19.74±3.1%
AGS	51.28±12.6%	17.34±2.2%	9.80±1.1%
PC-3	**91.45±3.7%**	**89.75±4.7%**	**87.5±2%**

Figure 2: Suppression of LNCaP cell growth upon administration of MU-Gold



Significance of MU-Gold's G1 Phase Cell Cycle Specificity

In order to evaluate the ability of MU-Gold to inhibit the growth of cancer cells through the inhibition of cell cycle progression, we have carried out *in vitro* experiments in tissue culture upon incubation of cancer cells with 20-40 μg/mL of MU-Gold. Cell cycle distribution of HCT-15 cells, derived from human colon carcinoma, was evaluated to clarify the mechanism of growth inhibitory effect of MU-Gold. Results of cell-cycle analysis for the HCT-15 cell lines confirmed that the proportion of cells in the G1 phase increased from 4% for untreated control cells to 22% for the cells cultured for 48h with 20 μg/mL MU-Gold [Table 2]. The increase in the proportion of cells in the G1 phase was even greater at 53% when cells were cultured with 40μg/mL of MU-Gold [Table 2].

Table 2: Percent of HCT-15 cells in each cell-cycle phase

Cell Cycle Phase	Control	Cells incubated with 20μg/ml MU-Gold	Cells incubated with 40μg/ml MU-Gold
G1	4%	22%	53%
S	89%	70%	36%
G2+M	7%	9%	12%

These results demonstrate that MU-Gold can inhibit the growth of colon cancer cells by means of G1 phase cell cycle arrest. It is well known that cell proliferation, quiescence, differentiation or programmed cell death (apoptosis) are decided within the G1 phase of the cell cycle, thus making it a critical phase in determining the cell's fate. Therefore, disrupting or malfunctioning of cell cycle control within G1 phase has been recognized as the most important biochemical phenomenon for tumor progression and tumorigenesis. The ability of certain small molecules to control machinery within G1 phase has now provided exciting new opportunities with hopes of developing new types of drugs efficacious against refractory cancers. In fact, several agents that are "cytostatic" but affect cell cycle within the G1 phase are being currently reexamined under clinical investigations. Our discovery of a new water-soluble gold compound (MU-Gold) with novel G1-targeting antitumor properties provides the first example of metal-based agents with cell cycle inhibitory properties. It is the G1 phase cell cycle specificity of MU-Gold that distinguishes this compound from the other gold-based chemotherapeutic agents and also from

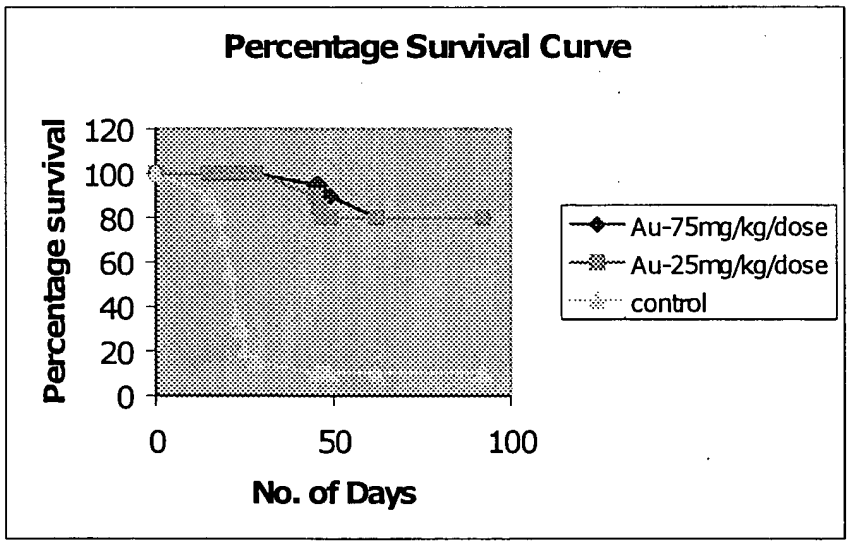
that of *cis*-Platin. This is significant because MU-Gold, through its ability to arrest cell cycle in the G1 phase, may provide a new molecular basis to fight hormone refractory or androgen independent prostate cancer.

In vivo Effects in Tumor Bearing Mice

We have also examined the *in vivo* antitumor activities of MU-Gold, using mice administered syngenic Meth/A cells intraperitoneally (i.p). Survival curves shown in Figure 4 demonstrated that administration of 25mg/kg subcutaneously (s.c.) (and up to 125mg/kg) in tumor bearing mice resulted in remarkable survival. The MU-Gold was administered s.c. on the first, third and fifth day from the time of tumor inoculation. It is important to recognize that >98% of the animals treated with MU-Gold survived beyond six weeks and the survival percentage remained at 84% even at 90 days (Figure 3). The control group which was injected with saline alone showed >95% mortality within six weeks (Figure 3).

Administration of MU-Gold in doses of up to 125mg/kg (375mg/kg total dose) i.p did not result in deaths of tumor bearing mice. In fact the **LD_{50} of MU-Gold in rats is approximately 375mg/kg**. No acute toxicity of this compound was observed at the doses tested (up to 375mg/kg) in animals. Also, at the doses of MU-Gold tested so far, no serious hematologic or renal side effects were noticed in mice.

Figure 3: Survival curves of tumor-bearing Balb/C mice treated with MU-Gold s.c.



(20 animals were used in each experimental set)

Preliminary Efficacy Studies on Solid Tumor Suppression

In order to test and provide proof of principle that MU-Gold is effective in shrinking solid tumors in mice models, we have carried out therapeutic efficacy studies in a limited group of SCID mice implanted with human prostate cancer PC-3 xenografts. SCID mice 20-25 g in weight were inoculated subcutaneously in the bilateral flank with 0.1mL injectates containing approximately 5 X 10^6 human prostate PC-3 cancer cells. Animals with palpable tumors measuring 3.5-5.5 mm diameter were selected for therapeutic efficacy studies. SCID mice with PC-3 xenografts were divided into three groups of five animals. The control group was injected with saline i.p, whereas the other two groups were treated with 40mg/Kg/day and 60 mg/Kg/day of MU-Gold *via* i.p administration of the aqueous solution of the drug. The treatment was continued for 10 days. The tumors were measured once a week for 60 days and the tumor volume was calculated as length x width x height x 0.5236. Body weight of the animals were recorded weekly. Animal survival was measured daily.

As shown in Figure 4, MU-Gold at 40 mg/Kg and 60 mg/Kg powerfully suppressed growth of PC-3 tumors in SCID mice. A significant inhibition of tumor growth in the two treated groups was observed within two weeks after the start of the experiment. The magnitude of tumor inhibition was dose dependent. The treatment with MU-Gold induced a persistent regression of all tumors in the groups given 40 mg and 60 mg of MU-Gold. Therapy with 40 mg and 60 mg of daily doses of MU-Gold for 10 days significantly reduced the mean final tumor volume to 44.20 ± 1.31 and 17.5 ± 1.1 mm^3 respectively at 50 days, as compared with controls, which measured 101.0 ± 3.5 mm^3. These limited, therapeutic efficacy studies although demontrate "**Proof of principle**" that MU-Gold shrinks solid prostate tumors, more detailed and systematic studies must be performed to evaluate dosing regimens to determine the effective therapeutic efficacy of MU-Gold in SCID mice with PC-3 tumors.

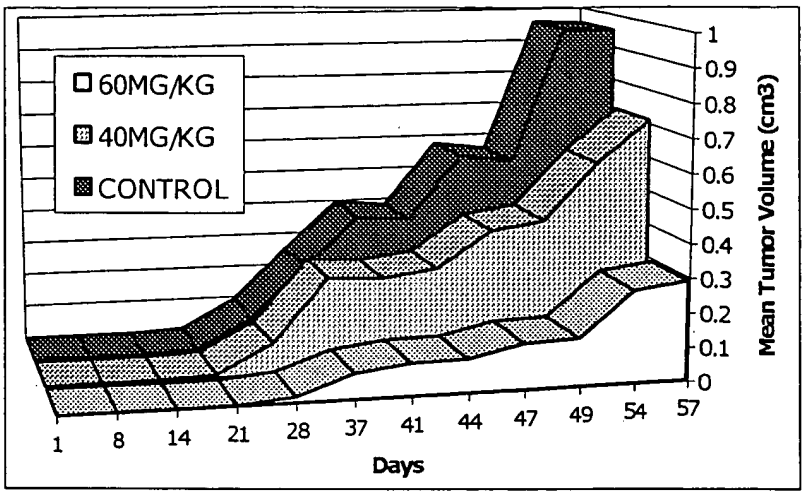


Figure 4: Effect of Varying Doses in MU-Gold in Shrinking Solid Tumors of SCID Mice Implanted with Human Prostate Cancer Xenography

No significant changes in the body weight within the treated animals were observed during the entire 60 days of observation. In the control groups, the tumors began to ulcerate at 60 days and therefore, animals were sacrificed. The white blood count and the platelet counts were normal in the treated animals.

In summary, our preliminary data suggest strongly that MU-Gold is a novel chemotherapeutic agent with unprecedented G1 cell cycle specificity. Its remarkable efficacy in suppressing growth of androgen dependent and androgen independent prostate cancer cells, *in vitro*, effectiveness in promoting survival time of tumor-bearing mice and its apparent low toxicity demonstrate that MU-Gold is a promising new chemotherapeutic agent for use in the treatment of colon, gastric and prostate cancer.